REPORT OF AID ASSIGNMENT TO SAIGON, VIETNAM, MAY 12-JULY 3, 1973

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My responsibilities in Vietnam were divided between two separate subject areas:

1. Consultant on the establishment of a national feed analysis laboratory
2. Observe and make appropriate recommendations on the swine industry of South Vietnam

Observations of the swine industry were interspersed throughout the time in country. Travels associated with the swine study included driving distance around Saigon, the Con Tho and Long Xuyen area in the south and the Nha Trang and Dalat area in the north.

Section A -- Completion of the National Feeds Analysis Laboratory

The construction of the feeds laboratory was in excess of 90% towards completion when I arrived on May 15, 1973; however, the laboratory could not be put in operation when I left on July 3. The contractor did allow us to occupy the building although it was not entirely completed. Laboratory glassware and analytical equipment were in position and ready for use. Electrical transformers had not been installed; therefore, electrical analytical equipment could not be used.

The laboratory was constructed in the shape, number of rooms and size proposed originally by Dr. J. Kastelic. However, the architecture of the exterior has caused difficulty for space utilization within the building. Doors are placed in inefficient locations. Windows or louvered areas span the entire length of building. This limits the use of space immediately inside the window. In one room (Room C) the architect proposed

a digestion hood immediately inside the windows, obviously for simpler venting design. The proximity of digestion flasks to window or louvered areas would result in inefficient, variable or incomplete digestion. It was necessary to move the digestion units away from the window and proposed site of the hood. We did not install the hood but used venting equipment shipped with the digestion apparatus.

Each room of the analytical laboratory has a louvered section (at least eight square feet) immediately above the desk tops. The success and reliability of an analytical laboratory is dependent on the repeatability of results. Wind currents and dust blowing through these louvers would minimize any chance of accumulating accurate data. I proposed that all of these louvers be replaced with glass.

To close these louvers will increase the heat and humidity load within the building. I suggest that air conditioners be installed in rooms where the analytical determinations are being made (Rooms A, B and C at present).

The laboratory benches were not designed for the height of Vietnamese technicians. The counter tops are much too high for convenience. The counter tops are made of one-inch tiles imbedded in concrete. This surface tends to be uneven and exceedingly hard. Either condition contributes to increased glass breakage.

The recommended analytical procedures as given to the director of the laboratory are printed in the Appendix.These include sample collection, preparation, procedural sequence and appropriate calculations. The procedures include analyses for crude protein, ether extract, crude fiber, ash, calcium and phosphorus. These criteria are most often shown on a feed label and are required by feed laws in many countries.

Analyses of other nutrients may be necessary from time to time to cope with particular problems. Such a problem exists at the present time. Much of the fish meal in Vietnam is adulterated with salt added as a preservative before shipping and processing. High levels of sodium and chloride cause diarrhea in swine and could cause death in poultry. Analysis for either sodium or chloride would allow identification of the adulterated sources. The data obtained could be used by the processors of high quality fish meal to promote their product. I have proposed that a chloride procedure be added to the list of analytical procedures and have discussed a procedure with the director of the laboratory.

These analyses will meet the initial needs of the laboratory in supporting a proposed national feed law. Procedures for the quantitative determination of individual amino acids, trace minerals or vitamins should be implemented as the laboratory and demand develop.

There is one area of equipment availability that should be investigated. The United States Army and Air Force had a practice of determining wear on parts and equipment by sampling the oil from engines and transmissions and measuring for metals with atomic absorption spectrophotometry. Many spectrophotometers were in use in Vietnam and I feel certain many others were in storage. The next major piece of analytical equipment I would suggest would be an atomic absorption spectrophotometer. Is it possible that such equipment is now available from surplus or other means of transfer? Mr. Thong has had experience in operating a spectrophotometer.

A National Feed Analysis Laboratory is essential to the regulatory role in maintaining feed quality standards throughout the country. I see an equally important role for this laboratory in the area of education. The

laboratory can be used to demonstrate to livestock producers and feed mill managers the importance of nutrient adequacy of feeds. I would hope that a procedure could be established and producers encouraged to send ingredients and feeds to the laboratory for analysis. Some producers I visited accepted what they were mixing or feeding with faith in following directions rather than understanding why they were combining these ingredients. We have the same problem in Illinois and have attempted through various means to increase understanding. My background and interests force me to look at such a laboratory with a different bias than a chemist might. I view a feeds analysis laboratory as a referee, but I also believe that it provides a quality control system for the benefit of the livestock manager.

The magnitude of the regulatory role in time may increase so that all the capability of the laboratory will be needed for the single responsibility. In the meantime the laboratory can provide a valuable service in education and information.

The young man designated as director of the National Feeds Laboratory is very competent. Mr. Thong was well trained while earning his Master's degree at the University of Minnesota. This degree training was augmented by the training received at the Minnesota State feeds laboratory. When I left Vietnam I felt confident that Mr. Thong could ably complete items he and I left undone because of the lack of electricity.

There was interest in Saigon for sending several additional technicians to the United States to train to work in this laboratory. At the present time there are one or possibly two technicians with training in the United States that have been designated to work in the laboratory. I recommend that no more than two technicians with such training be used at a time. Frankly, I view some disadvantage in having too many technicians

with highly advanced training in analytical procedures in the one laboratory. Additional staff could be trained for specific assignments right at the National Feeds Analysis Laboratory.

Section B -- Review of Swine Industry in South Vietnam

During the period May 15 through July 3 I visited numerous swine farms in South Vietnam. On one three-day trip into the Delta I observed farms in the areas of Can Tho and Long Xuyen. I spent approximately a week studying units in the general area of Nha Trang, Phan Rang and Dalat. At various times during my stay in Vietnam I traveled to swine farms within driving distance of Saigon. These farms varied in volume from a few finishing pigs to 12,000 head marketed per year.

All of these hogs were raised in confinement. One could not help but be tremendously impressed with the quality of management and care practiced. The hogs were maintained on concrete and kept exceedingly clean. In most units the floors were washed or flushed daily. I cannot comment on the source or the quality of the wash water.

There is considerable variation in the understanding of nutrition among swine producers in Vietnam. The concept of using vitamin-trace mineral premixes has been well presented in South Vietnam. Most farms I visited formulated their own diets and on each of these farms vitamin-trace mineral premixes were used as part of the diet. American corn and soybean meal constitute a large segment of the swine feed. This is changing rapidly with the increased planting of milo and corn for animal feed. The striking figure is the increase in milo production in the past three years from 4,000 to 10,000 to 30,000 hectares. There remains hesitancy on the part of some Vietnamese swine producers to pay as much for milo as for corn. Corn is priced 50% greater than milo. Feeding trials are needed to compare Vietnam-

grown milo with corn. Normally corn and milo are used interchangeably in swine rations without influencing swine performance.

On several farms there is still the feeling that pigs need fresh green vegetable leaves (greens) for good performance. "Greens" are excellent sources of some vitamins (not all) but contain very little other value for the pig. At a point in history pigs receiving "greens" grew more rapidly. However, as indicated most Vietnamese farmers use a complete vitamin mixture. Both sources of vitamins are not necessary. Since the "greens" are incomplete, I encourage the continued use of vitamin premix and not the greens. They aren't harmful but do not improve the nutrition of the pig.

Probably the greatest benefit of greens is that they keep the pigs occupied. In so doing they may reduce tail biting. Tail biting results from a stress or as some have stated a social disease resulting from boredom. It is a severe problem in Vietnam. The incidence can be reduced by the following means. First, we recommend cutting off two-thirds of the tail at birth. The stub of the tail thickens as it heals. It is difficult for another pig to attack the short tail and it is less easily damaged. Tail biting can be minimized by providing other outlets for the pigs' time and energy. Producers have used pieces of pipe or chain that pigs carry around or chew, bowling balls for the pigs to push or as in this case greens to chew. Quite often tail biting is increased in pigs suffering from a physical stress such as heat or cold or being fed an inadequate diet. I would recommend producers clip the tails on their pigs at birth.

While in Vietnam I heard much about the merits of opaque-2 corn. On page 27 of the 1972 Report to the Ambassador by the Director of USAID, Vietnam, I read that except for vitamins, high-lysine corn is a balanced diet in itself. I don't believe that statement. The report does not state

the specie that the report refers to, but I think readers would presume swine and poultry.

Incidentally, we use the term opaque-2 corn since it implies at least a genetic variation from regular corn. High lysine is an unfortunate choice of terms. The most severe amino acid deficiency in opaque-2 corn is lysine whether describing for man or swine. The term low-lysine corn would be just as appropriate in some cases.

Introducing the opaque-2 gene into our corn population changes the concentration of at least three amino acids: lysine, tryptophan and leucine. The magnitude of change is variable. For instance, we obtained 18 opaque-2 corn samples selected across Illinois from the 1972 crop. The lysine varied from 0.27% to 0.41% and averaged 0.33%. Regular corn contains 0.25 to 0.28% lysine. A study of similar design conducted in Iowa reported the same mean value (0.33%) for lysine. Opaque-2 corn samples with average values would provide 38, 46 and 55% of the lysine requirement for the weanling, growing and finishing pig, respectively. Corn is also woefully lacking in calcium, phosphorus and certain trace minerals for swine or poultry.

I could find no one in Vietnam that had obtained an analysis for lysine although many cultured it. I obtained two samples of opaque-2 corn grain raised in Vietnam and had them analyzed. The results were:

Variety	Lysine	Tryptophan
	%	%
Thai Composite	0.65	0.12
Western White	0.30	0.10

These represent a single sample of each variety; therefore, conclusions cannot be drawn. The values do demonstrate the variability

noted for opaque-2 corn. If further analyses confirm the value of the Thai Composite variety, this is the most valuable variety of opaque-2 corn reported. The 0.65% lysine would meet the amino acid needs of finishing swine. To conduct agronomic studies with opaque-2 corn and suggest nutritional implications without reporting actual lysine values is a rather useless exercise and may be misleading to swine and poultry producers.

Considerable data on the nutritional value of native feed sources needs to be accumulated and then disseminated to swine producers. There appears to be much confusion on the substitutive value of feedstuffs such as rice bran, bananas and cassava. The corn-soybean meal diet works very well in the States and has been transplanted to Vietnam. There seems to be a reluctance to use other ingredients. In actuality there may be numerous reasons why other ingredients may be much more appropriate for that area both in terms of economics and the use of domestic materials.

I intend to search the literature and provide NLTC (Saigon) and USAID with data I can find on these products. The studies I have seen on rice bran have not been at all definitive in terms of feeding value. For instance, substitution was made for corn in most trials with no consideration or credit for the shift in amino acid pattern. With the tremendous quantity of rice bran available in Vietnam I would encourage the support of nutritional research by appropriate agencies for well-planned and executed nutritive value studies.

At the present time there are extensive studies underway in South America in which both cassava and bananas are being evaluated as energy sources for swine.

The final feed ingredient I am concerned about is fish meal. Fish meal contains the highest concentration of lysine of the natural feed-

stuffs available today. As long as we are fortifying cereal grains, lysine will be the amino acid of greatest concern. Yet, I find much reluctance by swine producers to use the product. They are certain that most fish meal will cause diarrhea in pigs. As indicated in the report on the National Feeds Analysis Laboratory much of the fish meal is laden with salt. Since most fish meal processing plants are located inland, the companies have added salt to the fish to prevent putrefaction during transit and drying. The levels of salt will cause diarrhea.

All fish meal is not adulterated. However, the swine producer is not able to differentiate between sources and therefore bids a low price on all fish meal. Vietnam has great potential for producing fish meal, yet production will not expand markedly until this condition is corrected.

a. I have proposed that the National Feeds Analysis Laboratory establish a chloride analysis procedure to differentiate between quality and adulterated products.

b. I encourage experimentation with acetic or propionic acid as preservatives during shipment instead of salt. Formaldehyde has also been used as a preservative in such a situation. However, formaldehyde is more likely to reduce protein quality.

c. Salt should not be added to diets containing this fish meal. (This seems obvious, but I found producers adding salt to these diets.) The adulterated fish meal could be mixed with quality products when they are identified.

d. I encourage companies that are producing quality fish meal to promote their product on the basis of low salt content.

e. I encourage the animal husbandry personnel in each province to disseminate the information on fish meal.

Time of Weaning

Most swine producers in Vietnam do not wean pigs until eight or nine weeks of age. This age is proper for some producers. Other producers with more complete facilities should consider weaning at younger ages. Milk production peaks at about four weeks of age and by eight weeks there is very little milk being produced. Pigs will be heavier at eight weeks if removed from the sow at a younger age and placed on a dry diet. This presupposes that a diet for young pigs is available. This may not always be possible so I recommend weaning at six or seven weeks and using a less formidable diet.

There is another advantage to weaning at earlier ages. Sows normally come into heat (estrus) about six days after the pigs are weaned. Obviously to wean quicker allows the producer to rebreed more quickly. Confinement facilities to be profitable must be efficiently utilized which means keeping them full.

Ventilation

Confinement appears to be an integral part of modern swine production in Vietnam. The building construction consists primarily of a concrete floor, fence of different materials, an open area above the fence and a roof over most of the unit. Most fence material along the periphery of the building as well as dividing fences are solid concrete. These solid walls varying from two and one-half to four feet in height are a serious deterrent to ventilation. The pig has no normal sweat glands, therefore, other means of heat loss are exceedingly important. Therefore, in Vietnam with an average temperature in the Saigon area of 85° F. and a high humidity, ventilation is most critical. Concrete construction is economical in Vietnam compared with the alternatives, but openings in the concrete can be included quite effectively.

Solid fence may be an important deterrent to reproductive efficiency. In the case of the breeding herd early heterosexual experience is needed for effective matings. Recognition of the opposite sex through open fence may be sufficient to enhance sex drive in males and estrus expression in females. Some breeding problems I observed could have been due to this complete isolation. Environmental heat, which is increased in those sweat boxes, will suppress libido and causes the development of abnormal, weak or dead sperm in boars.

Medication

I saw many examples of overmedication in both swine and poultry. Antibiotics cannot correct for inadequate management or incomplete diets. The continued feeding of extremely high levels of antibiotics have never been recommended by manufacturers. Drugs other than antibiotics also are used unjustifiably. The best example was the producer that injected calcium gluconate into all sows in the farrowing house regardless of the time of lactation. Whatever responses may be obtained with dairy cattle has never been demonstrated in sows. Producers need guidance in choosing feed additives and injectable therapeutics and selecting the appropriate level.

Water for Young Pigs

I found resistance to providing water continuously for all animals. In particular, there is the feeling that the nursing pig doesn't need water other than milk. In reality the pig receiving only milk is just narrowly in water balance. Any diarrhea will put the pig in negative water balance. All young pigs should have access to water. Performance will be increased and protection will be given againstthe dehydration associated with diarrhea.

Use of Farrowing Crate or Guard Rails

I saw extremely solidly constructed farrowing pens. In most pens there was no protection of the pigs in the form of guard rails or entire farrowing crates. These protective devices will save more than a pig per litter. More space was wasted in the farrowing pens than in any other confinement pen. Extremely long, narrow pens (approximately 4 x 18 feet) with solid concrete wall panels had been built on many farms. No more than 40 square feet is needed by the sow and litter.

Breeding Stock

The American breeds of swine have been spread through Vietnam in a relatively short period of time. The use of American breeds on native stock has brought about rapid changes in the carcasses. On many farms the native stock has simply been marketed. Even native swine crossed with American breeds were seldom seen. Carcass improvement has placed a premium on the purebred breeds of American origin. There needs to be more advantage taken of crossing the American breeds for resulting hybrid vigor in the sows, boars and market pigs.

Summary

In spite of the critical remarks in these paragraphs I was most impressed with the scope of biological and economic management existing on the Vietnamese swine farms. It is most interesting to find aspects of production in Vietnam as advanced as any place in the United States while on the same farms there are obvious errors of omission. Their willingness to incorporate modern technology suggests that as the time and opportunity permit other improvements will be considered.

BGH/sef
10/11/73

APPENDIX

GENERAL NOTES ON FEED ANALYTICAL PROCEDURES

The procedures outlined herein represent approved methods routinely employed throughout the world in feed analysis laboratories and in all possible instances they are based on those adopted by the Association of Official Agricultural Chemists, Washington, D. C. The few modifications in these methods were made for two principal reasons:

(A) The modified procedures have been in use in the laboratories in the Department of Animal Science for many years and thoroughly checked and validated using the criteria of simplicity, consistency in reproducability of results and accuracy. They do not require the use of expensive or unstable reagents and measuring devices.

(b) They are designed to meet the needs of technicians who are obliged to perform routine analyses on a large variety of feeds and feed ingredients without recourse to the use of a specialized procedure for each particular type of feed or ingredient.

It may be noted that the wet ashing procedure shown for ashing feeds, and/or materials high in fat preparatory to the calcium and phosphate determination can be used for virtually any vegetable or animal material. The $Mg(NO_3)_2$ ashing procedure for the phosphate determination is on the other hand suitable for materials or mixtures of materials that contain organic materials, mineral supplements and such resistant materials as rice and cottonseed hulls.

The most effective wet ashing procedure in current use is, however, the one requiring the use of nitric acid in conjunction with perchloric acid. This very useful procedure was not recommended because experience has shown that in the hands of the average laboratory technician and in the absence of expensive properly designed fume hoods, explosions and fires are ever

present and dangerous occurrances. Other notes about the analytical procedures in this brochure appear in the outlines given for them.

GENERAL PROCEDURES AND COMMENTS CONCERNING THE ANALYSIS OF FEED SAMPLES

1. The most important factor in feed analytical work is that feed samples used for analysis be a truly representative part of the original material and when obtained that they be identified, labeled and dated in a positive easily recorded manner and available for reference at any time.
2. Stored feed samples are best held in moisture proof containers and stored in an insect and rodent free storage facility. Perishable feed materials may require drying before storage but in such cases the moisture content of the sample must be recorded for future reference.
3. Feed analysis records must be kept in a secure place and original record entries should never be removed from the laboratory unless noted and released by a responsible supervisor.
4. All calculations shall be verified by both the analyst and another individual and so initialed and dated on all work sheets. Calculation rechecks shall be done beginning with original data recorded at the time the analyses were performed.
5. All work sheets will be given to the supervisor immediately in completion of the analytical work on the feed samples or series of feed samples.
6. Discrepancies in samples, in the procedure used or any unusual characteristics of feed samples should be recorded on the margin of the work sheet. Extensive notes about feed samples may be recorded on the back side of the work sheet. Do not record comments about feed samples on detached sheets of paper.

7. The supervisor of the Feeds Analysis Laboratory will maintain the final rechecked laboratory data on each feed sample analyzed, in appropriate ledgers and file folders.
8. Only feed analysis methods approved by the supervisor will be used. No modifications may be applied to any procedure without approval of a responsible supervisor.
9. All standard reagent solutions and measuring devices will be rechecked at periodic intervals to insure that they are stable and capable of producing consistently accurate results.
10. A generous supply of reference standard feed samples representing a cross section of feeds and feed ingredients routinely analyzed in the laboratory must be maintained under the laboratory director's supervision. These should be submitted under coded designations to laboratory personnel for specific analytical procedures at periodic intervals to validate the results of the procedures. The data so obtained must be consistently recorded with dates when the analyses were done and the records held in only the laboratory director's files.

Such laboratory control measures particularly if verified occasionally and independently in another feeds analysis laboratory are indispensible to effective feed quality control work.

SAMPLING PROCEDURES

1. This is the first and most crucial step in feed analyses work. The taking of samples must without exception be done by a competent reliable disinterested individual whenever there is some liklihood for legal consequences or control.

The following chart indicates how samples may be taken. See also AOAC 22.001, page 327, 10th Edition, 1965.

Amount of Feed	Sample No.*	Sample Size**
1 bag	4-5 core samples	1-2 kg.
1-4 bags at least	1 core from each bag; minimum 5 cores	1-2 kg.
1-10 bags	1 core from each bag	1-2 kg.
10 bags or more	1 core from random selection of 10 bags	1-2 kg.
Bulk feed storage	10 cores from different region	2-5 kg.

Samples which cannot be sampled with a probe should be sampled by other means, e.g., grab samples taken from different regions.

* Coring tube used in feed sampling with slots and pointed end.

**Sample size may be reduced by piling feed sample into a cone shaped heap and quartering until about 500 grams are obtained. Mixing feed samples on clean paper is a good practice providing separation of fines is avoided.

2. The feed samples should be immediately transported to the laboratory site in moisture proof containers, ground to pass a sieve with about 1 mm diameter openings and repackaged in a clearly identified manner in a moisture proof container for subsequent storage in the feed analysis laboratory. Plastic bags which will hold a kilogram of feed are cheap, convenient and easily marked with felted instant drying marking pens. Marked and used storage bags must be discarded in order to minimize mistakes in identification.

BASIC FEEDS

Microscopy: (see 22.055, pages 337-340, AOAC, 10th Edition, 1965)

The quantitative analytical chemical procedures for assessing the quality of feeds and feed ingredients are not adequate for the detection of specific sources of chemical constituents of feeds.

For example the Kjeldahl procedure provides a convenient means for measurement of the quantity of nitrogen in a mixed feed but it cannot provide the means for identifying the nitrogen sources in the feed. Unscrupulous individuals have been known to add unhydrolyzed feather meal, leather scraps, urea, hair or other nitrogenous materials to mixed ground feeds in order to meet minimum declared N x 6.25 values specified by feed control laws. In other instances additions of cheap phosphate compounds largely or not available to animals have been made to satisfy the minimum content of P in a ration.

The detection of feed adulterants such as weed seeds, rice, cotton or cereal grain products with low feed value is best done with the aid of a microscope. Indeed routine basic feed microscopy is an essential aspect of feed quality control work.

This procedure is especially invaluable for the detection and identification of many classes and types of adultrants in feeds or mineral and organic constituents which if present in feeds can be seriously questioned on nutritional grounds.

It is generally accepted that one of the best means for curbing unscrupulous practices in the feed industry is to establish the capacity to do routine chemical and microscopic feed examinations and to vigorously prosecute violators of the feed quality regulations. This is usually sufficient to deter further violations.

However, we must always be mindful that even a complete chemical and microscopic examination of a feed does not give us all the information we need. Obviously, chemical analyses must always be coupled with studies of the biologic responses of the animal. Without such studies we cannot yet accurately predict the digestibility of the various fractions of feeds, or the efficiency with which the nutrients are absorbed and utilized.

THE PROXIMATE ANALYSIS OF FEEDS

PROCEDURE FOR DETERMINATION OF DRY MATTER

PREPARATION OF DRYING CUPS FOR DRY MATTER

Number, clean and dry 2 aluminum cups with matching covers* for each sample to be run. Put cups in 105° C. oven for 1-2 hours. Remove and place cups in a dessicator and cool for 1 hour. Weigh and record weight for each cup and store in any container with cover until ready to use. Cups should be well numbered so that they can be easily identified at weighing time.

SAMPLE PREPARATION

Take weighing cup, add enough of feed sample with spatula or small clean spoon to give the desired weight of about 2-3 grams. Weigh to 0.1 mgm. and carefully record weight of cup and feed sample in the cup. Repeat this procedure until two or three cups with weighed feed samples are obtained. Place unconvered cups and samples in 105° C. oven and heat for exactly 5 hours. Remove to dessicator, cool 1 hour and weigh cups after covers are replaced. Save samples for ether extract.

NOTE: (1) Do not handle cups with hands anytime after washing--use crucible tongs

(2)*Covers need not be used if exposure to air is minimized during weighing and cups are stored in air tight containers. Dried feed samples intended for further analysis must be kept in dessicators or other moisture proof containers.

EQUIPMENT, GLASSWARE, REAGENTS REQUIRED

1. Dessicators and dessicant
2. Aluminum weighing cups with covers
3. Drying oven with heat regulator to maintain 105° C. over several hours
4. Chemical balance capable of weighing to 0.1 mg.
5. Small stainless steel spoons or spatulas
6. Diethylether for washing weighing cups

ADDITIONAL REFERENCES

See in vacuo moisture determination procedures, section 22.003, page 327, AOAC, 10th Editdon, 1965.

NOTE: Preparation of bulk grains and dry forages requires use of feed grinding equipment such as the Wiley mill.

DETERMINATION OF DRY MATTER IN FEEDS

Feed Sample laboratory identification _____

Feed sample No.* _____

RESULTS:

	Feed Sample Number		
	No. _____	No. _____	No. _____
BEFORE DRYING			
Weight of feed sample + cup	_____	_____	_____
Weight of empty cup	_____	_____	_____
I. Difference (sample weight)	_____	_____	_____
AFTER DRYING			
Weight of dried feed sample + cup	_____	_____	_____
Weight of empty cup	_____	_____	_____
II. Difference (dry sample)	_____	_____	_____

% dry matter weight
II divided by I x 100

Feed Sample No. _____ _____ x 100 = _____ %

Feed Sample No. _____ _____ x 100 = _____ %

Feed Sample No. _____ _____ x 100 = _____ %

Average = _____ %

Analyst _____ Date _____

Checked by _____ Date _____

*Note suggestion: Give each separate feed sample a number followed month-day-year received as shown.

DETERMINATION OF DRY MATTER IN FEEDS

Feed Sample laboratory identification Complete Chick Starter

Feed sample No.* N.L.C. 231-6-15-70

RESULTS:

	Feed Sample Number		
	No. 231a	No. 231b	No. 231c
BEFORE DRYING			
Weight of feed sample + cup	16.206	15.102	16.018
Weight of empty cup	13.036	12.107	13.058
I. Difference (sample weight)	3.170	2.905	2.960
AFTER DRYING			
Weight of dried feed sample + cup	15.753	14.604	15.609
Weight of empty cup	13.036	12.107	13.058
II. Difference (dry sample)	2.717	2.497	2.551

% dry matter weight
II divided by I x 100

Feed Sample No. 231a $\frac{2.717}{3.107} \times 100 = 85.7\ \%$

Feed Sample No. 231b $\frac{2.495}{2.905} \times 100 = 86.0\ \%$

Feed Sample No. 231c $\frac{2.551}{2.960} \times 100 = 86.2\ \%$

Average % H_2O $= \frac{86}{14}\ \%$

Analyst _____ Date _____

Checked by _____ Date _____

*Note suggestion: Give each separate feed sample a number followed month-day-year received as shown.

PROCEDURE FOR THE DETERMINATION OF ASH IN FEED

Preparation of Equipment

Heat and clean 50-100 cc. beakers in 105° C. oven 2 hours, put in dessicator, cool 1 hour and weigh. Number and obtain empty weights for each beaker.

Sample Preparation

Place 2 grams of dried feed samples in clean and dry beakers and weigh. (If feed samples are wet, put in 105° C. oven overnight.) Place beakers with feed samples in muffle furnace and ash overnight at 550° C. Turn muffle furnace off and allow to cool to about 125° C., put samples in dessicator and cool 1 hour and weigh. (If it is too inconvenient to get samples out of oven until it gets below 100° C., it will be necessary to put in 105° oven for 1-2 hours before putting in dessicator.)

EQUIPMENT, GLASSWARE, REAGENTS REQUIRED

1. Ashing crucibles or 50-100 ml. pyrex beakers (beakers are less expensive and equally good)
2. Ashing oven (muffle furnace) with controls to regulate, rate of heating and maintenance of maximum heating temperature.
3. Drying oven (for very wet samples)
4. Dessicators and dessicant.
5. Chemical weighing balance capable of weighing to 0.1 mg.

See also ash determination section 22.010, page 328, OAOC, 10th Edition, 1965.

PROCEDURE FOR THE DETERMINATION OF ASH IN FEEDS

Feed Sample Laboratory Identification_____

Feed Sample No._____

	Feed Sample No._____	Feed Sample No._____	Feed Sample No._____
BEFORE ASHING			
Weight of empty beaker + sample	_____	_____	_____
Weight of empty beaker	_____	_____	_____
I. Difference (Weight of feed)	_____	_____	_____
AFTER ASHING			
Weight of beaker + ashed sample	_____	_____	_____
Weight of empty beaker	_____	_____	_____
II. Difference (Weight of ash)	_____	_____	_____

% ash in feed

No._____ = _____ x 100 = _____% ash

No._____ = _____ x 100 = _____% ash

No._____ = _____ x 100 = _____% ash

Average = _____% ash

Analyst_____ Date_____

Checked by_____ Date_____

THE PROXIMATE ANALYSIS OF FEEDS
PROCEDURE FOR THE DETERMINATION OF CRUDE PROTEIN; DETERMINATION OF TOTAL ORGANIC NITROGEN BY THE KJELDAHL METHOD

PREPARATION OF EQUIPMENT FOR THE CRUDE PROTEIN DETERMINATION

Laboratory Facilities

The Kjeldahl room must be well ventilated by a high capacity wall fan to remove heat and fumes (sulfur trioxide) which may escape into the room during the digestion of feed samples. The laboratory area in which this procedure is carried out should be equipped with a safety shower equipped with overhead fast water release valve which can be quickly opened by a pull chain, easily reached.

The concentrated sulfuric acid used in Kjeldahl digestions should be dispensed from carefully assembled equipment to prevent spillage or splatter of acid or breakage of acid containing vessels.

Kjeldahl flasks used in this determination need to be washed only with tap water (if it is low in nitrogen bearing materials) and rinsed once with distilled water (to effect economy in use of distilled water rinsing of large flasks).

Wear safety glasses when working with Kjeldahl flasks.

DIGESTION OF SAMPLES FOR CRUDE PROTEIN DETERMINATION

Add about 15 grams of the catalyst mixture to each flask.* Place an amount of sample containing 20-60 mg N in each 500 ml Kjeldahl flask (for feeds about 2-3 grams). Add 25 ml concentrated H_2SO_4 so as to wash down into the bottom of the flask any of the sample caught in the neck of the Kjeldahl flask.

If a large amount of low nitrogen materials is used add additional acid for each gram of sample added; about 10 ml of H_2SO_4 for each additional gram of feed sample.

*See subsequent notes.

Place flasks on digester and adjust heaters to low heat and turn on blower. Some materials will froth or foam at the start. In this case turn heat off for a short while, then turn heat on again when foaming subsides. Digest slowly until visible feed materials (organic matter) have disappeared. Cool to room temperature, wash down neck and sides of flask with 50-60 ml distilled water. POINT FLASK AWAY FROM YOU DURING ALL ADDITIONS OF MATERIALS TO THE ACID MIXTURE. Replace flasks on heater and digest slowly 4 hours. Cool to room temperature. Add boiling chips, 3 drops paraffin oil, 300 ml distilled water and mix well.

DISTILLATION OF DIGESTED SAMPLES: (Boric acid procedure for trapping distillate nitrogen as NH_3).

Adjust all the required heaters to give an even low heat and then turn off until distillations are started.

Meanwhile turn water valve to circulate water in condensers. Add 50 ml boric acid solution containing the indicator to each receiving flask and place flask in position with receiving tube in the boric acid. WEAR SAFETY GLASSES. Add to each Kjeldahl flask 85 ml of NaOH-thiosulfate mixture allowing the solution to run down the neck of the flask VERY SLOWLY, so that it will run under the acid layer, being careful that no mixing is done. Connect flask to distilling head firmly. DO NOT PUSH ON BOTTOM OF FLASK. When all flasks are in position, shake each one vigorously to mix thoroughly and turn on heater.

This SHAKING AND TURNING ON HEATERS must be done rapidly so flasks do not cool and suck back boric acid before all heaters are turned on.

There will be sufficient heat to start boiling with no danger of any boiling over, if heaters have been properly adjusted.

After about 75 ml of distillage has been condensed, receiving flasks should be lowered so that receiving tube is no longer in contact with the distillate

and distillation continued until flasks begin bumping or 150 ml have been distilled over. Then shut off heat, disconnect Kjeldahl flask from distilling head, wash tube in receiving flask with neutral water, remove receiving flask and cover until ready to titrate. When Kjeldahl flasks are cool enough to handle, remove and wash thoroughly with tap water, rinse with distilled water and hang upside down in carts to drain.

TITRATION

Fill burettes to 0.00 or record reading at start of titration for each titration and allow acid to drip slowly, stirring constantly with glass rod until end point (grey) is reached. Record titration values, obtained. Dump contents and turn erlenmeyer upside down on self to drain.

Remove identification from both titration and Kjeldahl flasks. Do not leave flasks on digestion rack overnight or for any extended period when you are going to be away. Shut off all water and heaters and close clamp between burettes and standard acid supply as soon as you finish. Wipe up all spilled acid and alkali and flush the sink thoroughly.

See also 2.043 and 2.044, page 16; 22.001, page 327 of the Official Methods of Analysis of the Association of Official Agricultural-Chemists, 10th Edition, 1965.

REAGENT SOLUTIONS FOR NITROGEN DETERMINATION

Boric acid solution: 900 gms boric acid added to 20 liters distilled H_2O.

Mix into solution. Add 95 cc indicator (methyl/red; methylene blue).

Catalysts* (1) 0.7 grams H 0 + 15 grams K_2SO_4 or Na_2SO_4 or

(2) commercial product as directed.

*Amount for each Kjeldahl flask.

Sodium thiosulfate solution: 4000 gms sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) per 10 liters distilled H_2O (mixed into solution).

Sodium hydroxide-NaOH (alkali): 15 liters concentrated NaOH solution plus 2 liters sodium thiosulfate solution. Make to volume of 17 liters total volume.

STANDARD H_2SO_4 ACID SOLUTION (FOR TITRATION)

NOTE: The following procedure will provide a 0.13-0.15 normal acid solution.

PROCEDURE

290 gms concentrated H_2SO_4 (Baker's or equivalent) are added to total volume of 40 liters distilled H_2O. Mix very thoroughly.

The following standardization method is used to determine normality factor of the acid. The Na_2CO_3 is heated first in a drying oven set at 150° C. for 5 hours. It is then taken and put in a dessicator for one hour to bring to room temperature. Then it is weighed by difference and placed in a separate Erlenmeyer flask. Five Erlenmeyer flasks containing 0.2 to 0.3 gms to nearest mg or Na_2CO_3 weighed by difference are used to titrate to a neutral color. 50 cc of neutral H_2O is added to each flask, and then 4 drops of indicator (see below). A 50 ml burette is then filled with about 30 ml of H_2SO_4 and titrated against the Na_2CO_3. The calculation shown below is used to determine the normality of the acid.

Indicator Solution: Methyl red, 1.0 gm
Methylene blue, 0.4 gm) 1000 ml 95% alcohol

Calculation of Normality of Acid:

$$\frac{\frac{\text{Gm } Na_2CO_3}{106}}{\text{ml titration}} \times 1000 = \text{Normality}$$

NOTES ON KJELDAHL DIGESTION PROCEDURE

One may use other catalysts for digesting feed samples, for example, one containing copper sulfate (10 grams per sample) made up of:

(a) 98 grams potassium sulfate Kjelgest brand and 2 grams of copper sulfate, best grade and powdered.

Note that if mercury is used as a catalytic agent sodium thiosulfate must be added to the NaOH used to neutralize the acid digests in order to "tie up" the mercury prior to distillation.

One may use a standard acid solution to trap the NH_3 during the distillation of feed sample digests and titrate the excess standard acid with standard NaOH; see 2.044, page 16, AOAC Procedures, 10th Edition, 1965.

The procedure presented in this outline has the advantage that only one standard acid solution is needed. Furthermore, this procedure takes advantage of the sharp end point one can obtain in the titration with the mixed methyl red-methylene blue indicator.

MOISTURE DETERMINATION
CRUDE NITROGEN ANALYSIS

All weight and titration data must be recorded directly in a record book to avoid copying errors and to facilitate checking.

APPARATUS, GLASSWARE AND CHEMICALS for the determination of crude protein in feeds:

1. Kjeldahl digestion apparatus
2. Kjeldahl distillation apparatus
3. Methyl red
4. Methylene blue
5. Commercial grade sulfuric acid
6. Commercial grade NaOH
7. C.P. Grade NaOH
8. C.P. Grade HCl
9. Boric acid
10. Glass beads
11. Paraffin oil
12. Potassium sulfate, Kjelgest brand
13. C.P. Copper Sulfate (optional)
14. C.P. Sodium Thiosulfate and Metallic Mercury or H O
15. Acid Potassium Phathalate for standardizing NaOH solutions (optional)
16. 500 ml Erlenmeyer flasks
17. 500 ml Kjeldahl flasks
18. 50 ml volumetric burettes; large (25 liter) storage vessels for concentrated NaOH, 10 liter vessels for standard acid and NaOH solutions and boric acid solution
19. Heavy shatter proof 25 liter glass storage vessel for concentrated sulfuric acid
20. Assorted volumetric pipettes and flasks for measuring and standardizing reagents, chemical balance, Wiley mill.

COMPUTATIONS; EXAMPLES:

Factor for determining % nitrogen in feed sample.

Assume in standardization of acid that:

1. Weight Na_2CO_3 used 0.2083 grams
2. Titration of acid = 13.55 ml

$$\text{Normality of acid} = \frac{\frac{0.2083}{106}}{13.55} \times 1000 = \frac{1.965}{13.55} = 0.1450$$

Calculation for % nitrogen in feed sample.

$$\frac{0.014 \times (\text{net titration for distillate}) \times \text{Normality of acid} \times 100}{\text{Weight of sample in grams}}$$

1. Factor (constant for each lot of standard acid)

   0.014 x Normality of acid x 100 = Factor

or 0.014 x 0.1450 x 100 = 0.203

See example shown on following page for calculations involved in the determination of Crude Protein Content of a feed.

CRUDE PROTEIN ANALYSIS

Feed Sample Laboratory Identification Mixed Chick Feed

Feed Sample No. 282-6/13/70

Dry weight of feed sample used 1.4900 Date 3/1/70

1. Normality standard acid solution 0.1450 Date 3/1/70
2. Factor for standard solution* 0.203 Date 3/1/70
3. Blank titration for N in reagents 0.020 Date 3/1/70

Burette Readings

	Feed Sample		Blank	
end	23.10	ml	23.30	ml
start	0.15	ml	23.10	ml
difference	22.95	ml	0.20	ml

corrected or net titration = 22.75 ml

$$\% \text{ Nitrogen in Feed Sample} = \frac{\text{Factor} \times \text{ml titration}}{\text{sample weight}} = \frac{0.203 \times 22.98}{1.4900}$$

= 3.13 % Nitrogen

% Crude Protein in Sample = % Nitrogen x 6.25 = 3.13 x 6.25=19.56 %

Name of Analyst _____ Date _____

Checked by _____ Date _____

* To facilitate calculation 0.014 x normality of acid x 100 = factor to compute % N in feed sample.

CRUDE PROTEIN ANALYSIS

Feed Sample Laboratory Identification_____

Feed Sample No._____

Dry weight of feed sample used_____ Date_____

1. Normality standard acid solution _____ Date_____
2. Factor for standard solution* _____ Date_____
3. Blank titration for N in reagents_____ Date_____

	Burette Readings	
	Feed Sample	Blank
end	_____ ml	_____ ml
start	_____ ml	_____ ml
difference	_____ ml	_____ ml

corrected or net titration = _____ ml

% Nitrogen in Feed Sample = $\frac{\text{Factor x ml titration}}{\text{sample weight}}$ = _____

= _____ % Nitrogen

% Crude Protein in Sample = % Nitrogen x 6.25 = _____ %

Name of Analyst_____ Date_____

Checked by_____ Date_____

*To facilitate calculation 0.014 x normality of acid x 100 = factor to compute % N in feed sample.

THE PROXIMATE ANALYSIS OF FEED

THE DETERMINATION OF ETHER EXTRACT (E.E.)

Laboratory Facilities

Well ventilated laboratory

Absence of open flames

Smoking must not be permitted

PREPARATION OF ETHER EXTRACT GLASSWARE

Take 3 clean, numbered ether flasks for each sample. Wipe thoroughly with clean cloth to remove dust and fingerprints and, with crucible tongs, put in 105° C. oven for about 2 hours. Remove and put in dessicator. Cool 1 hour and weigh. Store in covered container until needed.

PROCEDURE

Take feed samples saved from dry matter determination. Transfer to aluminum thimbles. Put in soxhlet extractor. Place small piece of cotton on top of sample to prevent spattering. Fill flask about two-thirds full of low-boiling petroleum ether (skelly-solve), or diethylether, connect to rack and extract for 8-16 hours. Condensation rate will determine time required. Rapid condensation of 4-5 drops per second will hasten rate of extraction and hence may be completed in 8 hours. Slow condensation such as 2-3 drops per second may require 12-16 hours extraction time.

Check frequently to keep ether supply sufficient to insure good siphon action. Disconnect soxhlet at end of extraction procedure and remove, with special tongs, thimble and sample. Reconnect flask and drive ether from flask to soxhlet, emptying soxhlet at intervals before it siphons back to flask. Do not, at any time, pour ether from flask. Put flask on steam bath (VERY LOW TEMPERATURE) for

a few minutes to remove ether fumes. Wipe flask thoroughly with cheese cloth and put in 90° C. oven for 1 1/2 to 2 hours, then in dessicator for 1 hour and weigh sample. See procedures 22.033, AOAC, 10th Edition, 1965.

EQUIPMENT AND GLASSWARE REAGENTS FOR THE DETERMINATION OF ETHER EXTRACT

1. Ether extraction apparatus (Soxhlet type)
2. Ether extraction flasks
3. Aluminum extraction thimbles
4. Flask cork seals or gaskets

   (These are easily damaged and a couple of dozen of them as spares should always be on hand.)
5. Low boiling petroleum ether (skelly-solve) or diethylether
6. Dessicator and dessicants
7. Chemical balance
8. Steam bath

THE DETERMINATION OF ETHER EXTRACT

(Crude Fat)

Feed Sample Laboratory Identification_____

Feed Sample No._____

BEFORE EXTRACTION

	No._____	No._____	No._____
Weight of dried feed samples	_____	_____	_____

AFTER EXTRACTION

Weight of flask + extract sample	_____	_____	_____
Weight of flask (empty-dry)	_____	_____	_____
Difference (extract)	_____	_____	_____

% Ether Extract in Feed Samples

No._____ = _____ x 100 = _____%

No._____ = _____ x 100 = _____%

No._____ = _____ x 100 = _____%

Average = _____%

Analyst_____ Date_____

Checked by_____ Date _____

THE DETERMINATION OF ETHER EXTRACT

(Crude Fat)

Feed Sample Laboratory Identification: Mixed Swine Feed

Feed Sample No.: 293- 2/18/70

BEFORE EXTRACTION

	No. 293a	No. 293b	No. 293c
Weight of dried feed samples	3.112	3.402	3.268

AFTER EXTRACTION

Weight of flask + extract sample	61.328	65.419	62.337
Weight of flask (empty-dry)	61.080	65.137	62.087
Difference (extract)	0.248	0.282	0.250

% Ether Extract in Feed Samples

No. 293a = $\frac{0.282}{3.112}$ x 100 = 8.0 %

No. 293b = $\frac{0.282}{3.402}$ x 100 = 8.3 %

No. 293c = $\frac{0.250}{3.268}$ x 100 = 7.6 %

Average = 7.9 %

Analyst _____ Date _____

Checked by _____ Date _____

PROCEDURE FOR THE DETERMINATION OF CALCIUM IN FEEDS

Livestock rations containing mineral supplements will not have similar amounts of calcium. Layer rations may contain as much as 3% of calcium while swine rations may contain 0.8%.

PREPARATION OF FEED SAMPLES

A convenient and inexpensive ashing vessel is 100-150 ml pyrex beakers. Clean and dry by heating in a 100-105° C. oven as many pyrex beakers as are needed. Cool, number and weigh beakers and store in any container with cover until required.

Place about 2 grams of finely ground feed sample into beakers and weigh to nearest milligram.

ASHING FEED SAMPLES

Place beakers containing feed samples and ash overnight at 550° C. in muffle furnace.

Turn muffle furnace off and allow to cool to about 120° C. Place beakers containing ashed feed into a dessicator for 1-2 hours.

Remove from dessicators and weigh. One may use feed samples ashed for total ash content (see procedure for ashing feeds).

PREPARATION OF ASHED SAMPLES FOR CALCIUM DETERMINATION

1. Add 40 ml HCl (1 + 3) and a few drops of concentrated HNO_3 to ash and bring to boil.
2. Cool and transfer to 250 ml volumetric flask, dilute and make up to mark.
3. Pippette 100 ml into a 250 ml pyrex beaker and dilute to about 100 ml with distilled H_2O.
4. Add 2 drops of methyl red indicator.
5. Add drop by drop NH_4OH solution (1 + 1) until color changes to brownish-orange (pH about 5.6).

NOTE: If too much NH_4OH is added, add drop by drop HCl (1 + 3) until orange color appears.

6. Add 2 drops of HCl (1 + 3). Color should change to pink (pH 2.5 to 3.0)
7. Dilute to about 150 ml with distilled H_2O.
8. Place beaker on hot plate and bring to boil.
9. Add slowly with constant stirring, 10 ml of hot saturated solution of ammonium oxalate $(NH_4)_2\ C_2O_4$.

NOTE: If color should change from pink or red to orange or yellow after adding ammonium oxalate add drop by drop HCl (1 + 3) until color changes to pink.

10. Filter solution through quantitative filter paper after allowing solution as prepared to step 9 to stand overnight. Keep beaker for step 12.

NOTE: This step is important since it is essential that the calcium in the ash solution be precipitated as well formed calcium oxalate crystals.

11. Wash precipate collected on filter paper several times with NH_4OH (1 + 50) to remove excess ammonium oxalate from calcium oxalate precipate.
12. Place filter paper in original beaker and add a mixture of 125 ml of H_2O and 5 ml concentrated H_2SO_4.
13. Heat solution to 70° C. or above on hot plate.
14. Add slowly with stirring 0.1 N $KMnO_4$ solution until first pink color persists.

NOTE: Color will fade in a few minutes as filter paper is present.

15. Run blank on test run without ash sample.
16. Calculate percent calcium.

GLASSWARE AND REAGENTS FOR CALCIUM DETERMINATION

1. Hot plates
2. Volumetric burette (50 ml)
3. 250 ml pyrex beakers
4. 100-150 ml pyrex beakers for ashing

5. Funnels
6. Quantitative filter paper
7. Volumetric pipettes (25 and 10 ml)
8. Dropper pipettes (small)
9. HCl (C.P. grade)
10. HNO_3 (C.P. grade)
11. NH_4OH (C.P. grade)
12. $(NH_4)_2\ C_2O_4$ (C.P. grade)
13. Methyl red indicator

REAGENTS

1. HCl solution (1 + 3)

   Add 250 ml of reagent grade concentrated HCl acid to 750 ml of distilled H_2O.

2. NH_4OH solution (1 + 1).

   Add 500 ml concentrated NH_4OH to 500 ml of distilled H_2O.

3. NH_4OH solution (1 + 50)

   Add 10 ml NH_4OH to 500 ml distilled H_2O.

4. Saturated $(NH_4)_2\ C_2O_4$ solution

   Add 42 grams of $(NH_4)_2\ C_2O_4$ to 1000 ml of distilled water.

5. Methyl red indicator.

   Dissolve 1 gram of methyl red indicator in 200 ml ethyl alcohol (95%).

6. Dilute H_2SO_4 solution. Add cautiously 5 ml concentrated H_2SO_4 into 95 ml of distilled H_2O.

STANDARD REAGENTS

1. $KMnO_4$ (C.P. grade)
2. $CaCO_3$ (C.P. grade)
3. Sodium oxalate $Na_2C_2O_4$ (C.P. grade)

1. Sodium Oxalate Standard Solution

   (a) Weigh 6.700 grams of $Na_2C_2O_4$ on clean dry watch glass and transfer to 1000 ml volumetric flask. Make up to mark with distilled H_2O. This solution is a 0.1 normal solution of sodium oxalate and is to be used to standardize the $KMnO_4$ standard solution.

2. $KMnO_4$ solution 0.1 Normal

   This reagent requires careful preparation and the precautions listed must be observed if accurate results in the calcium titration are desired.

   (a) Dissolve 3.3 grams of dry $KMnO_4$ in one liter of distilled water and allow to stand two days in a clean glass stoppered bottle.

   (b) After standing, siphon* off the first 50 ml of solution and discard. Continue siphoning solution into a clean bottle but do not remove or siphon the last 2-5 cm of reagent in the bottom of the bottle which will be discarded.

*Use clean glass tubing since $KMnO_4$ reacts with rubber, filter paper or other kinds of organic matter.

   (c) The $KMnO_4$ is standardized against standard $Na_2C_2O_4$.

3. Calcium standard

   (a) Weigh 0.2500 grams of primary standard grade of $CaCO_3$ on clean dry watch glass and transfer carefully into 1000 ml volumetric flask.

   (b) Dissolve $CaCO_3$ with a few ml of HCl (1 + 1).

(c) Make up to volume (1000 ml) with distilled H_2O.

This solution will contain 0.1 mg of calcium per ml. This solution may be used as an internal standard to check the calcium determination procedure.

STANDARDIZATION OF $KMnO_4$ REAGENT

1. With the aid of a volumetric pipette introduce exactly 25 ml of $Na_2C_2O_4$ standard solution.
2. Add 25 ml of dilute H_2SO_4 (mix 5 ml of concentrated H_2SO_4 into 95 ml of H_2O before use).
3. Fill 50 ml burette with $KMnO_4$ solution and run in rapidly 22 to 23 ml of $KMnO_4$.

NOTE: If $KMnO_4$ solution is less than 0.1 N, more than 22 to 23 ml may be needed; if greater than 0.1 N, less than 22 to 23 ml should be used.

4. Allow solution to stand until the $KMnO_4$ is decolorized and then heat to 50° to 60° C.
5. Continue to add $KMnO_4$ solution slowly and with gentle stirring allowing each drop to decolorize before adding another.

The end point of this titration is reached when a faint pink color remains for about 30 seconds.

EXAMPLE FOR STANDARDIZATION

(a) Amount of standard 0.1 Normal $Na_2C_2O_4$ used = 25 ml

(b) Total amount of $KMnO_4$ used in titration = 24.5 ml

Normality of $KMnO_4$ x 25.5 = 25 x 0.1

Normality of $KMnO_4 = \frac{25 \times 0.1}{24.5} = 0.102$

Such a standard solution of 0.102 N $KMnO_4$ = 0.102 x 20 = 2.04 mg of calcium.

Based on 1 ml, 1.0 N $KMnO_4$ is equivalent to 20 mg of calcium.

PROCEDURE FOR THE DETERMINATION OF ASH IN FEEDS

FOR CALCIUM DETERMINATION

Feed Sample Laboratory Identification_____

Feed Sample No._____

	Feed Sample No.____	Feed Sample No.____	Feed Sample No.____
BEFORE ASHING			
Weight of empty beaker + sample			
Weight of empty beaker			
I. Difference (Weight of feed)			
AFTER ASHING			
Weight of beaker + ashed sample			
Weight of empty beaker			
II. Difference (Weight of ash)			

% ash in feed

No.____ = ____ x 100 = ____ % ash

No.____ = ____ x 100 = ____ % ash

No.____ = ____ x 100 = ____ % ash

Average = ____ % ash

Analyst_____ Date_____

Checked by_____ Date_____

Determination of Calcium in Feeds

Feed sample laboratory identification - mixed swine feed

Feed sample No. 311 - 3/18/70

Weight of dry feed in sample - 2.501

Aliquote taken - 100 ml from total volume - 250 ml

Fraction in titrated sample - 2.5

Normality of $KMnO_4$ standard - 0.052* date - 3/18/70

Factor for $KMnO_4$ standard - 1.04 Mg Ca date - 3/18/70

Blank titration for Ca in reagents - 0.25 date 3/18/70

* Adjust to .05 N if calcium content feed is low or use larger aliquote of feed sample

Burette Readings

	Feed Sample	Blank
End	10.60 ml	10.85 ml
Start	0.05 ml	10.60 ml
Difference	10.55 ml	0.25 ml

Corrected $KMnO_4$ titration - 10.30 ml

Mg Ca in titrated sample = 10.30 x 1.04 = 10.71 mg

Grams Ca in titrated sample = $\frac{10.71}{1000}$ = 0.0108 grams

Grams Ca in feed sample = 0.0108 x 2.5 = 0.027 grams

% Ca in feed sample = $\frac{0.027}{2.501}$ x 100 = 1.08%

Analyst_____ Date_____

Checked by_____ Date_____

10. Add 5 ml concentrated HCl and heat to dryness on steam bath.
11. Moisten residue and add 2 ml concentrated HCl and 25 ml distilled H_2O and warm on steam bath.
12. Transfer contents of beaker into a filter paper fitted funnel set in a 250 ml volumetric flask.
13. Continue rinsing beaker with distilled water and pour through filter. Four or five successive 10 ml lots of distilled water for rinsing the flask and washing residue, if any, on filter paper is sufficient.
14. Add enough distilled water to bring contents of flask to 250 ml mark and mix contents thoroughly.

DILUTION OF SAMPLE FOR COLORIMETRIC MEASUREMENT

1. Remove 25 ml of solution from 250 ml flask and add to 100 ml volumetric flask.
2. Add sufficient distilled H_2O to make up to 100 ml mark.

This solution will represent 1/1000 of the original feed sample on per ml basis.

PROCEDURE FOR THE COLORIMETRIC DETERMINATION OF TOTAL PHOSPHORUS IN FEEDS

1. Place 4 ml aliquot of diluted ashed sample (see above) into 10 ml graduated tube.
2. Add
   (a) 1 ml ammonium molybdate solution and let stand for a few minutes
   (b) 1 ml hydroquinone solution
   (c) 1 ml sodium sulfite solution
   (d) distilled water to make up to 10 ml mark
3. Mix thoroughly

4. Let stand for 30"

5. Transfer enough sample to colorimeter absorption tube to fill to 3/4 level

NOTE: Amounts needed will be determined by the size of colorimeter absorption tube in use. Some hold 2 ml while other types may hold more. Read absorption value against reagent blank set at 100% transmission; that is a tube containing reagents used but not containing sample. This is normally done by carrying two beakers through the entire procedure to which feed samples have not been added.

STANDARD CURVE FOR DETERMINING TOTAL PHOSPHATE CONCENTRATIONS in feeds using the colorimetric procedure.

STANDARD PHOSPHORUS SOLUTION

1. Weigh 0.4394 grams of dry reagent grade KH_2PO_4 and transfer to a 1 liter volumetric flask.
2. Add distilled water to 1000 ml mark and mix thoroughly.

STANDARD SOLUTION FOR USE

Take 25.0 ml of above solution and transfer to 200 ml volumetric flask. Make up to mark with distilled water. This solution will contain 0.0125 mg of phosphorus per ml.

DETERMINATION OF TOTAL PHOSPHORUS IN FEEDS

Ashing of feed sample

Procedures for ashing feeds for total phosphorus determinations vary. Wet ashing of feeds with perchloric acid (AOAC procedure) is an excellent one but it is very hazardous unless it is carried out by well trained experienced laboratory technicians.

The following procedure for determining the total phosphorus of feeds has been used for many years in the laboratories of nutrition and nutritional biochemistry in the Department of Animal Science at the University of Illinois with excellent results. It is, moreover, a simple easily handled method for the determination for total phosphorus in all kinds of animal and plant materials. A wet ashing procedure is indicated for materials high in fat; see subsequent notes.

PREPARATION OF FEED SAMPLES

1. Weigh 1 to 2 gram feed sample and transfer to previously weighed, clean, dry 100-150 ml pyrex beakers.
2. Moisten sample with 1 ml $Mg(NO_3)_2$.
3. Add cautiously 1 ml of concentrated HCl.
4. Place flasks on steam bath until sample approaches dryness.
5. Add cautiously another ml of HCl.
6. Continue heating samples on steam bath until sample is completely dry.
7. Transfer beaker with sample to cold muffle furnace, turn on heat and ash for 6-8 hours at 550° C.
8. After ashing is completed turn off muffle furnace and allow beakers and ash to cool.
9. Add to cool beaker 5 ml HCl (1 + 4) to dissolve ash.

DETERMINATION OF CALCIUM IN FEEDS

Feed Sample Laboratory Identification_____

Feed Sample No._____

Weight of dry feed in sample_____

Sample taken _____ml from total volume of _____ml

Fraction in titrated sample = _____

Normality of $KMnO_4$ standard_____* Date_____

Factor for $KMnO_4$ standard _____ Date _____

Blank titration for Ca in reagents_____ Date _____

	Burette Readings	
	Feed Sample	Blank
end	_____ml	_____ml
start	_____ml	_____ml
difference	_____ml	_____ml

corrected $KMnO_4$ titration _____ml

mg Ca in titrated sample = _____ x _____ = _____mg

grams Ca in titrated sample = _____ x _____ = _____grams

grams Ca in feed sample = _____ x _____ = _____grams

% Ca in feed sample = _____ x _____ = _____grams

*Adjust to .05N if calcium content feed is low or use larger fraction of feed sample.

Analyst_____Date_____

Checked by_____Date_____

PREPARATION OF STANDARD CURVE

10 ml tube No.	Phosphate standard (ml)	Mg* phosphate	Ammonium molybdate (ml)	Hydro-quinone (ml)	Sodium sulfite (ml)	Distilled H_2O (ml)
1	1	0.0125	1	1	1	6
2	2	0.025	1	1	1	5
3	3	0.0375	1	1	1	4
4	4	0.050	1	1	1	3
5(Blank)	0	0	1	1	1	7

Set colorimeter at 100% transmission using tube 5, blank.

Read successively standard phosphate tubes 1 to 4 and record percent transmission for each dilution.

Plot percent transmission against each of the five phosphorus standards (0.0125 to 0.050 mg) on semi-log graph paper. One may use optical density values plotted on ordinary graph paper.

The values for the feed samples may then be read from the standard curve.

NOTES: Colorimeter should be set to allow transmission readings at a wave length setting of 660 millimicrons.

If feed samples give high transmission readings (above 70%) use 5-6 ml aliquots for colorimetric readings. If readings are 40% or below use less or 2 to 3 ml aliquots. The amounts of reagents used in the colorimetric procedure will remain the same. Differences in volume are made with distilled water to make final volume to 10 ml.

*The amounts of phosphate per tube may need to be less than shown depending upon the type of colorimeter used, sensitivity and size of absorption tube. If these amounts of phosphate are too high use smaller amounts such as a standard with .005 mg per ml. Adjust feed sample aliquots accordingly.

REAGENTS

1. Standard KH_2PO_4 solution: Dissolve 0.4394 gm of pure dry KH_2PO_4 and make up to a liter. 50 ml of this solution diluted to 200 ml gives a standard of which 2 ml = 0.05 mg P. or 25 micrograms per ml.*
2. NH_4 Molybdate solution: Dissolve 25 gm NH_4 molybdate in 300 ml H_2O. Dilute 75 ml of H_2SO_4 in 125 ml H_2O and add to NH_4 molybdate solution.
3. Hydroquinone solution: Dissolve 0.5 g of hydroquinone in 100 ml H_2O and add one drop of H_2SO_4 to retard oxidation. Put in brown bottle. Make up fresh with boiled and then cooled every week. Do not use after one week old.
4. Sodium Sulfite solution: Dissolve 200 g of Na_2SO_4 in H_2O. Make up to 1 liter and filter with Whatman 42-185 cms. Either keep this solution well stoppered or make up fresh each time.
5. $Mg(NO_3)_2$ solution: Dissolve 45 grams of $Mg(NO_3)_2$ in water to make up 100 ml of total solution. [May use $Mg(NO_3)_2 \cdot 6H_2O$].
6. Concentrated HCl: Dilute HCl (1 + 4); add 25 ml of concentrated HCl and mix with 100 ml distilled water.

APPARATUS AND GLASSWARE

1. Steam bath
2. Muffle furnace
3. 100-150 ml beakers for ashing
4. Colorimeter with absorption tubes
5. Pipettes and volumetric flasks

*Additional dilutions may be made as necessary.

WET ASH PROCEDURE FOR PHOSPHATE AND CALCIUM DETERMINATIONS

This procedure is used for tissue, urine, wet silage or any sample that has a high fat content.

1. Weigh beaker to be used.
2. Weigh or measure sample into beaker.
3. Add approximately 40 to 50 ml concentrated HNO_3.
4. Be sure sample does not foam. So leave off heat 1 or 2 hours to be sure.
5. Heat just below boiling point with beaker covered until nearly clear. If not fairly clear add 5 to 10 ml more concentrated HNO and evaporate to dryness. Wash down with demineralized water and let evaporate to dryness.
6. Add 40 to 50 ml 30% hydrogen peroxide and heat until the dried residue shows no traces of organic matter. Then wash again with demineralized water and let evaporate to dryness. Proceed to make up to volume with concentrated HCl used as needed to put sample in solution. Avoid excess HCl.

PROCEDURE FOR THE DETERMINATION OF ASH IN FEEDS FOR PHOSPHATE DETERMINATION

Feed Sample Laboratory Identification_____

Feed Sample No._____

	Feed Sample No._____	Feed Sample No._____	Feed Sample No._____
BEFORE ASHING			
Weight of empty beaker + sample	_____	_____	_____
Weight of empty beaker	_____	_____	_____
I. Difference (Weight of feed)	_____	_____	_____
AFTER ASHING			
Weight of beaker + ashed sample	_____	_____	_____
Weight of empty beaker	_____	_____	_____
II. Difference (Weight of ash)	_____	_____	_____

% ash in feed

No._____ = _____ x 100 = _____ % ash

No._____ = _____ x 100 = _____ % ash

No._____ = _____ x 100 = _____ % ash

Average = _____ % ash

Analyst_____ Date_____

Checked by_____ Date_____

PROCEDURE FOR THE DETERMINATION OF ASH IN FEEDS
FOR PHOSPHATE DETERMINATION

Feed Sample Laboratory Identification Chick Grower Ration

Feed Sample No. 281 - 5-16-70

	Feed Sample No. 281a	No. 281b	No. 281c
BEFORE ASHING			
Weight of empty beaker + sample	56.128	55.020	57.836
Weight of empty beaker	54.298	53.090	56.131
I. Difference (Weight of feed)	1.830	1.930	1.705
AFTER ASHING			
Weight of beaker + ashed sample	54.409	53.218	56.234
Weight of empty beaker	54.298	53.090	56.131
II. Difference (Weight of ash)	0.111	0.128	0.103

% ash in feed

No. a = $\frac{0.111}{1.830}$ x 100 = 6.07 % ash

No. b = $\frac{0.128}{1.930}$ x 100 = 6.63 % ash

No. c = $\frac{0.103}{1.705}$ x 100 = 6.04 % ash

Average = 6.24 % ash

Analyst _____ Date _____

Checked by _____ Date _____

PROCEDURE FOR THE DETERMINATION OF ASH IN FEEDS FOR PHOSPHATE DETERMINATION

Feed Sample Identification 281 a 5/16/70

Feed Sample Weight 1.830 grams

Feed Sample Ash Weight 0.111 grams

% Ash in Feed Sample 6.07 %

Dilution of feed ash (1) 250 ml

Dilution of feed ash (2) 25 ml to 100 ml

Dilution factor $\frac{1}{250} \times \frac{25}{100} = \frac{1}{1000}$

$= 1000$

Sample taken for P colorimetric readings

	ml Sample	O.D. Readings	P/Tube**	Per ml Basis
Tube 1	blank	---*		
2	1	0.18	15	15
3	2	0.38	31	15
4	3	0.55	46	15
5	4	0.70	58	14
				15

Average P content micrograms per ml;

Amount in original sample = (dilution factor) $\frac{1000 \times 15}{1000}$ = 15-mg

= .015 grams

Total P in feed sample

% P in Feed Sample

$\frac{.015 \times 100}{1.830 \text{ (weight of feed sample ashed)}} = 0.82$ % P

*For this calculation 0.1 O.D. units = 8.3 micrograms phosphate

See standard graph

Hence, tube 1 contains $\frac{0.18 \times 8.3}{0.1}$ = 15 micrograms P

tube 2 contains $\frac{0.38 \times 8.3}{0.1}$ = 31 micrograms P.

**in micrograms P/Tube

Use of P standard graphed on semi log scale vs % transmission.

Calculation P in Feeds

Assume 3 ml sample with dilution factor of 1000 (see previous calculations) gave transmission reading of 30%.

P in tube = 43 micrograms

P per ml sample = $\frac{43}{3}$ = 14 micrograms

Amount in feed sample (1.830 grams = $\frac{1000 \times 14}{1000}$ = 14 mg = 0.014 grams

P % in feed = $\frac{.014}{1.830}$ x 100 = 0.76 %

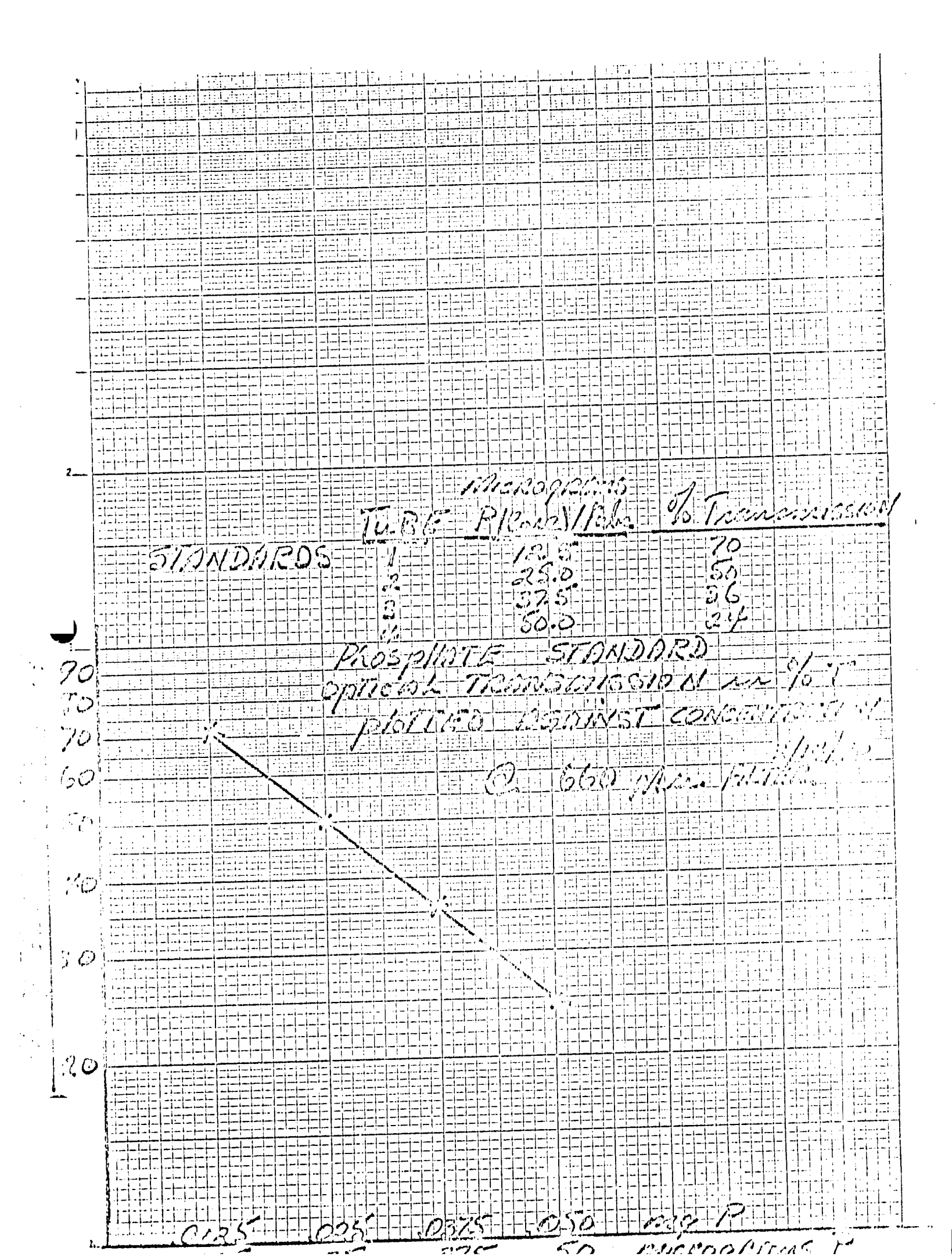
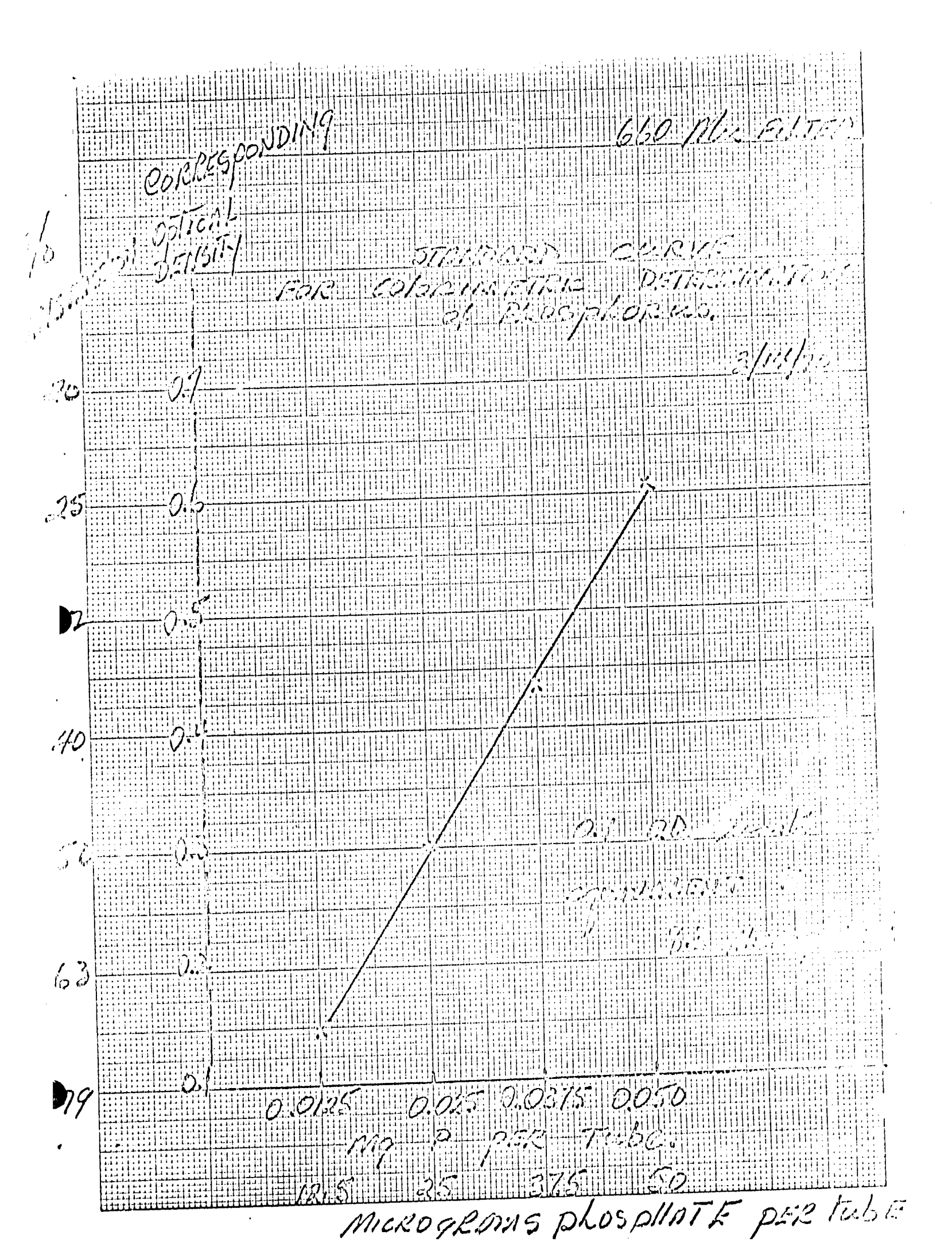

Micrograms
TUBE READING % Transmission
STANDARDS 1 15.5 70
2 25.0 50
3 32.5 36
4 50.0 24
PROSPITE STANDARD
Optical Transmission in % T
plotted against concentration
660 mμ Filter
70
60
50
40
30
20

TABLE 29-2: RELATION BETWEEN TRANSMITTANCY (T_s) AND OPTICAL DENSITY (D)

T_s (%)	D	T_s (%)	D	T_s (%)	D	T_s (%)	D
100	0.000	75	0.125	50	0.301	25	0.602
99	0.004	74	0.131	49	0.310	24	0.620
98	0.009	73	0.137	48	0.319	23	0.638
97	0.013	72	0.143	47	0.328	22	0.658
96	0.018	71	0.149	46	0.337	21	0.678
95	0.022	70	0.155	45	0.347	20	0.699
94	0.027	69	0.161	44	0.357	19	0.721
93	0.032	68	0.168	43	0.367	18	0.745
92	0.036	67	0.174	42	0.377	17	0.770
91	0.041	66	0.181	41	0.387	16	0.796
90	0.046	65	0.187	40	0.398	15	0.824
89	0.051	64	0.194	39	0.409	14	0.854
88	0.056	63	0.201	38	0.420	13	0.886
87	0.061	62	0.208	37	0.432	12	0.921
86	0.066	61	0.215	36	0.444	11	0.959
85	0.071	60	0.222	35	0.456	10	1.000
84	0.076	59	0.229	34	0.469	9	1.046
83	0.081	58	0.237	33	0.482	8	1.097
82	0.086	57	0.244	32	0.495	7	1.155
81	0.092	56	0.252	31	0.509	6	1.222
80	0.097	55	0.260	30	0.523	5	1.301
79	0.102	54	0.268	29	0.538	4	1.398
78	0.108	53	0.276	28	0.552	3	1.523
77	0.114	52	0.284	27	0.569	2	1.699
76	0.119	51	0.292	26	0.585	1	2.000

On photometers equipped with a linear scale reading from 0 to 100, D corresponds to the value of $2 - \log G$ where G is the galvanometer or microammeter reading relative to an initial setting at the 100 mark.


CORRESPONDING
OPTICAL
DENSITY
STANDARD CURVE
FOR COLORIMETRIC
DETERMINATION
OF PHOSPHORUS.
650 MU FILTER
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.0125
0.025
0.0375
0.050
Micrograms phosphate per tube